GENETICS

Implications of Sperm Chromosome Abnormalities in Recurrent Miscarriage

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Purpose: *Our purpose was to assess the existence of sperm chromosome abnormalities in recurrent pregnancy loss in an assisted reproduction program.*
Methods: *In this prospective study, 12 sperm samples from couples undergoing in vitro fertilization with two or more first-trimester spontaneous abortions were analyzed. Diploidy and disomy in decondensed sperm nuclei were assessed for chromosomes 13, 18, 21, X, and Y using two- and three-color fluorescence in situ hybridization.*
Results: *Sex chromosome disomy in sperm samples from recurrent abortion couples was significantly increased compared to that from internal controls (0.84% vs 0.37%). In a subpopulation of seven couples who underwent oocyte donation, mean frequencies for sex chromosome disomy (1%) were even higher and diploidy (0.43%) was also significantly increased.*
Conclusions: *These results suggest an implication of sperm chromosome abnormalities in some cases of recurrent pregnancy loss.*

KEY WORDS: unexplained recurrent miscarriage; chromosome abnormalities; sperm; nuclei; fluorescence in situ hybridization.

INTRODUCTION

Recurrent miscarriage is defined as three or more consecutive pregnancy losses, although there is a tendency to use a less rigorous definition of two or more abortions (1). This is a condition of great concern because, despite numerous studies, its etiology remains obscure (2) and the presence of an etiologic factor seldom suggests a conclusive diagnosis of the cause. Risk factors associated with recurrent pregnancy loss can be classified into six major groups: endocrine, anatomical uterine defects, infectious, immunological, genetic, and idiopathic (3).

Cytogenetic studies in first-trimester abortuses revealed that 50%–80% of concepti were chromosomally abnormal (4–8). In recurrent miscarriage patients, controversial results have been reported either suggesting that chromosomal abnormalities of the fetus occurred less frequently (9) or revealing significantly higher frequencies of aneuploid conceptions in these women (10).

Structural chromosome abnormalities were inheritated in only 3%–5% of the cases (10,11) and de novo chromosome defects may arise essentially at three developmental stages: gametogenesis, fertilization, and embryogenesis (12).

Aneuploidy results from meiotic nondisjunction during oogenesis and spermatogenesis, leading to abnormal gametes with disomies or nullisomies. Cytogenetic studies performed in unfertilized oocytes revealed a high percentage of aneuploidy (13% approximately), caused by maternal meiosis errors (13–15). On the other hand, the male contribution to recurrent miscarriage at the sperm chromosome level remains unexplored, and only peripheral blood karyotype has been performed. Meiotic abnormalities could arise throughout meiotic nondisjunction in the germ line

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(16,17), and thus a normal karyotype in blood cells does not exclude the presence of chromosomal abnormalities in spermatozoa. In this sense, a direct evaluation of sperm chromosome complements would be of interest for a better understanding of their role in habitual abortion.

The aim of this study was to assess to what extent sperm chromosome abnormalities were implicated in recurrent pregnancy loss in an assisted reproduction program. For this purpose, we analyzed a group of couples with two or more consecutive abortions and a negative workup for repetitive miscarriage. This group included patients with a normal ovarian response and patients who underwent ovum donation. The latter group led us to study a more specific subpopulation wherein oocyte factors were theoretically bypassed.

MATERIALS AND METHODS

Patients

Twelve couples of Caucasian origin with at least two previous consecutive spontaneous abortions in the first trimester were included in this study. A standard workup for repetitive miscarriage was performed and risk factors were discarded in all the cases. The evaluation protocol consisted of hysterosalpingography, endocrine analysis of thyroid hormones, follicle stimulating hormone (FSH), luteinizing hormone (LH), and prolactin (PRL), glucose test under fasting conditions, levels of anticardiolipin and antilupic antibodies, and parental karyotypes.

These couples were classified into two groups. Group I included five women with a "normal" ovarian response and 3.8 ± 0.6 previous spontaneous miscarriages, only one of them having a live-born infant; the mean age was 36.0 ± 1.6. Male partners (mean age, 37.6 ± 1.29) showed a normal sperm concentration, three of them with normal progressive motility percentages and the remaining two borderline. Group II was made up of seven women undergoing oocyte donation because of a low response to gonadotropins with 2.3 ± 0.2 previous abortions and a mean age of 41.1 ± 1.8. In this group the mean male age was 41.1 ± 1.7; five male partners were oligoasthenozoospermic, one was oligozoospermic, and the other one was asthenozoospermic. Morphology was not altered in any of the samples. In the control group, 14 samples from normozoospermic donors were analyzed and the results have been published previously (18,19). Protocols for oocyte donation and in vitro fertilization were carried out as described previously (20,21).

Fluorescence In Situ Hybridization (FISH) Protocol

Sperm samples were fixed in methanol: acetic acid (3:1) and processed for FISH analysis (22). Briefly, sperm nuclei were decondensed by slide incubation in 5 m*M* dithiothreitol (DTT) and 1% Triton X-100. Multicolor FISH was performed: triple-color FISH for chromosomes 18, X, and Y in one slide and dual-color FISH for chromosomes 13 and 21 in a second slide.

Centromeric DNA probes for chromosome 18 (locus D18Z1; CEP 18 Spectrum Aqua; Vysis Inc., Downers Grove, IL), chromosome X (locus DXZ1; CEP X Spectrum Green; Vysis Inc.), and chromosome Y (locus DYZ1; CEP Y Spectrum Orange; Vysis Inc.) were used for the triple-color FISH analysis. Locus-specific DNA probes for chromosome 13 (locus RB; LSI 13 Spectrum Green; Vysis Inc.) and chromosome 21 (loci D21S259, D21S341, and D21S342; LSI 21 Spectrum Orange; Vysis Inc.) were used for the dual-color FISH analysis. FISH incubation and detection were performed according to the manufacturer's instructions.

Analysis was done using an Olympus BX60 epifluorescence microscope equipped with a triple-band pass filter for DAPI/Texas Red/fluorescein isothiocyanate (FITC) and single-band pass filters for FITC, Texas Red, and Aqua blue. Sperm nucleus scoring was done strictly according to the criteria described by Blanco *et al.* (18). FISH slides of the control and the study cases were blindly analyzed by the same observer, to avoid biased results.

Data were compared using Fisher's exact test (InSat 2.01); $P < 0.05$ was considered statistically significant.

RESULTS

Hybridization and decondensation efficiencies were in each case higher than 99%. Haploidy, diploidy, and disomy for recurrent miscarriage patients ($n = 12$) are shown in Table I. In recurrent miscarriage patients, 10,129 spermatozoa were analyzed for chromosomes 18, X, and Y and 9317 spermatozoa for chromosomes 13 and 21. Sex chromosome disomy (0.84%) was significantly increased ($P < 0.0001$). However, autosomal disomy and diploidy frequencies did not reach statistical significance compared to those of controls.

Table I. Disomy and Diploidy Frequencies in Recurrent Miscarriage Patients*

	Recurrent abortion (n = 12)	Controls (n = 14)
No. sperm studied, 13/21	9,317	28,044
Haploidy, 13/21 (%)	9,113 (97.81)	27,867 (98.73)
Disomy, 13 (%)	9 (0.10)	—
Disomy, 21 (%)	18 (0.19)	91 (0.37)
No. sperm studied, X/Y/18	10,129	51,399
Haploidy, X/Y/18 (%)	9,937 (98.10)	50,823 (98.88)
Sex chromosome disomy (%)	85 (0.84)[a]	188 (0.37)[b]
Disomy, 18 (%)	3 (0.03)	48 (0.10)
Diploidy (%)	59 (0.30)	203 (0.25)

* Superscripts a and b = statistical difference between columns.

Results were also analyzed according to the origin of the oocytes (Table II). In group I (n = 5), there were statistical differences (P = 0.01) only in sex chromosome disomy, with a percentage of 0.61%. The mean frequency of sex chromosome disomy in group II (n = 7) was determined to be 1.00%. This figure showed a significant increase compared to that of the control group ($P < 0.0001$). The mean number of diploid cells in group I was similar to that in controls, but accounted for 0.43% in group II, and this value was again significantly different (P = 0.0008). Autosomal disomies for chromosomes 13, 18, and 21 were not significantly increased in either patients or groups.

There were significant differences between group I and group II: in sex chromosome disomy (P = 0.046) and, especially, in diploidy ($P < 0.0001$).

Individual results for each patient in group I are detailed in Table III. In group I, 4112 spermatozoa were analyzed for chromosomes 18, X, and Y and 3603 for chromosomes 13 and 21. As shown in Table III, sex chromosome disomy was significantly increased ($P < 0.05$) in two samples (Nos. 4 and 5); however, diploidy rates were not increased in any of them.

In group II (Table IV) a total of 6017 sperm nuclei was scored for chromosomes 18, X, and Y, and 5714 for autosomes 13 and 21. Two of the patients (Nos. 1 and 5) showed significantly increased frequencies for sex chromosome disomy, and three of them (Nos. 1, 2, and 5) for diploidy ($P < 0.05$).

DISCUSSION

In the present work, direct analysis of decondensed sperm nuclei revealed an increased incidence of chromosomal abnormalities in repetitive abortion couples. Disomy frequency for sex chromosomes was significantly increased in this group and diploidy rates were also increased in some of the samples. These findings suggest an implication of sperm chromosome abnormalities in recurrent pregnancy wastage.

To date, classic clinical investigations on the male partner of recurrent miscarriage patients have involved only paternal blood karyotyping (23) and evaluation of sperm parameters such as concentration, motility, and morphology (24), and direct sperm nucleus analysis has not been included.

A normal karyotype does not exclude the presence of chromosome abnormalities in spermatozoa. Such abnormalities could arise de novo in the germ cell line, and the most common anomaly observed was meiotic arrest with synaptic anomalies. Meiotic studies performed among 1100 infertile and sterile males revealed 4.84% of meiotic abnormalities in testicular biopsies

Table II. Disomy and Diploidy Frequencies in Recurrent Miscarriage Patients According to the Origin of Their Oocytes*

	Group I (n = 5)	Group II (n = 7)	Controls (n = 14)
No. sperm studied, 13/21	3603	5714	28044
Haploidy, 13/21 (%)	3526 (97.86)	5587 (97.77)	27687 (98.73)
Disomy, 13 (%)	5 (0.14)	4 (0.07)	—
Disomy, 21 (%)	10 (0.28)	8 (0.14)	91 (0.37)
No. sperm studied, X/Y/18	4112	6017	51399
Haploidy, X/Y/18 (%)	4045 (98.37)	5892 (97.92)	50823 (98.88)
Sex chromosome disomy (%)	25 (0.61)[a]	60 (1.00)[b]	188 (0.37)[c]
Disomy, 18 (%)	1 (0.02)	2 (0.03)	48 (0.10)
Diploidy (%)	8 (0.10)[d]	51 (0.43)[e]	203 (0.25)[f]

* Superscripts a, b, c, d, e, and f statistical differences between columns.

Table III. Disomy and Diploidy Frequencies in Patients with a Normal Ovarian Response (Group I)

	Patient No.				
	1	2	3	4	5
Concentration (million/ml)	82	55	70	82	97
% progressive motility	69	68	56	42	46
No. sperm studied (13/21)	517	512	1058	1000	516
Disomy, 13 (%)	1 (0.19)	1 (0.19)	2 (0.19)	1 (0.10)	0
Disomy, 21 (%)	0	1 (0.19)	4 (0.38)	3 (0.30)	2 (0.39)
No. sperm studied (X/Y/18)	508	528	1030	1029	1017
Sex chromosome disomy (%)	1 (0.20)	0	7 (0.68)	8 (0.78)*	9 (0.89)*
Disomy, 18 (%)	0	0	1 (0.09)	0	0
Diploidy (%)	3 (0.29)	0	2 (0.09)	3 (0.15)	0

* Statistical differences from the control group ($P < 0.05$).

which could arise throughout meiotic nondisjunction during spermatogenesis (16). Therefore, cytogenetic studies on spermatozoa are of great interest to assess their chromosomal constitution.

Evaluation of disomy for autosomes 13, 18, and 21 and sex chromosomes in recurrent abortion patients revealed a higher incidence of sex chromosome disomy. Although the prevalence of sex chromosome disomy is only 0.61% in group I and 1% in group II, several data indicate that moderate but significant increases in a given type of disomy are related to an increase in aneuploidy in the offspring (25). Similar findings have been reported on the incidence of different chromosomal abnormalities in sperm nuclei and there is evidence of chromosome-specific patterns of paternal nondisjunction, specifically for sex chromosomes (26) and autosomes 16 (27) and 21 (18, 26).

In our study, although an increased incidence of sperm chromosome abnormalities was observed in the recurrent miscarriage group, maternal contribution could not be completely ruled out, thus we classified the recurrent abortion population in two groups according to the origin of their oocytes. This approach led us to study a group of oocyte recipient patients (group II) in which maternal contribution could be theoretically discarded and sperm was analyzed as an "isolated" risk factor.

In group I, sex chromosome disomy was significantly increased, but the frequency of diploid sperm did not differ statistically. Chromosomal abnormalities

Table IV. Disomy and Diploidy Frequencies in Ovum Donation Patients (Group II)

	Patient No.						
	1	2	3	4	5	6	7
Concentration (million/ml)	9	0.1	3.5	3.5	10	11.5	60
% progressive motility	36	0	40	36	9	60	1
No.sperm studied (13/21)	1012	1023	1018	1065	579	510	507
Disomy, 13 (%)	0	0	0	1 (0.09)	2 (0.34)	1 (0.20)	0
Disomy, 21 (%)	3 (0.30)	0	1 (0.10)	1 (0.09)	2 (0.34)	0	1(0.20)
No. sperm studied (X/Y/18)	891	1022	1025	1022	1034	518	505
Sex chromosome disomy (%)	40 (4.50)*	2 (0.20)	5 (0.49)	2 (0.20)	9 (0.87)*	2 (0.39)	0
Disomy, 18 (%)	0	1 (0.10)	0	0	1 (0.10)	0	0
Diploidy (%)	19 (1.0)*	11 (0.53)*	2 (0.10)	6 (0.29)	11 (0.68)*	2 (0.19)	0

* Statistical differences from the control group ($P < 0.05$).

were not increased as markedly as in group II and maternal contribution to pregnancy wastage might be also considered in this group.

In group II, a higher incidence of disomy for sex chromosomes and diploidy was found compared to the control group. These differences were highly significant compared not only to controls, but also to group I, especially in diploidy.

Abnormal sperm morphology has been also associated with increased abortion rates (28), although some authors did not find a direct implication (24). Oligozoospermia has been suggested as another etiologic factor (29) and two hypotheses were proposed to explain these findings: a genetic etiology in poor-quality sperm samples and toxic sperm factors that attach to the oocyte possibly inhibiting secure implantation.

In our study, most of the samples in group I were normozoospermic, but in group II, six of seven patients were oligoasthenozoospermic and one was asthenozoospermic. This second group exhibited a higher incidence of diploidy and sex chromosome disomy as has been described previosly in oligoasthenozoospermic patients (25,30,31).

Our data support the idea of a genetic etiology, indicating that there is a trend toward a higher incidence of sex chromosome disomy and diploidy in these spermatozoa. The presence of toxic sperm factors cannot be ruled out and a larger number of patients would be required to confirm these preliminary data.

FISH is an accurate technique to detect the most common aneuploidies in decondensed sperm nuclei (25) and their introduction in an in vitro fertilization setting would be an effective approach in the management of repetitive abortion couples. If a higher incidence of specific chromosome abnormalities could be found, it would help in the choice of treatment strategies, such as insemination with donor sperm or preimplantation genetic diagnosis. In fact, the latter approach has already been used to avoid the transfer of an abnormal embryo to the uterus in recurrent miscarriage couples (32, 33).

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